Remarks

Claims 1-7, 10, 34 and 35 were pending in this application. Claims 1, 34, and 35 have been amended, new claims 36-40 have been added, and no claims have been canceled. Reconsideration of this application is respectfully requested in light of the above amendments and the following remarks.

Patentability of claims 1-7, 10, 34-40
Under U.S.C. § 103(a) in light of the
September 17, 2009 Examiner's Interview

Applicants respectfully submit that pending claims 1-7, 10, and 34-40 are allowable under U.S.C. § 103(a). Independent claims 1, 34, and 35 each require that mRNA transcripts encoded by GalNAcT and PAX3 marker genes are amplified and the levels of the mRNA transcripts encoded by the GalNAcT and PAX3 marker genes are detected. Similarly, independent claim 36 requires that mRNA transcripts encoded by GalNAcT, PAX3, MAGE-A3 and MART-1 marker genes are amplified and the levels of the mRNA transcripts encoded by the GalNAcT, PAX3, MAGE-A3 and MART-1 marker genes are detected. In each case, the levels of mRNA transcripts encoded by the marker genes from a first melanoma patient are compared to the levels of mRNA transcripts encoded by the marker genes from a second melanoma patient. The levels of the mRNA transcripts between patients are compared for the purpose of making a prediction regarding "metastatic melanoma recurrence, metastatic melanoma-free survival, overall survival, or combinations thereof." The present invention is directed to methods of determining the prognosis of melanoma patients. This is in contrast to each of the cited references which are directed to detection of melanoma cells.

In the September 17, 2009 Examiner's Interview (the Interview), the Examiner discussed in detail his reasoning regarding the patentability of the pending claims. The analysis proffered by the Examiner is as follows. The Examiner stated that the existence of marker genes related to melanoma is known in the prior art. The heterogeneous nature of cancer provides motivation to combine markers because such a combination will improve the ability to detect cancer cells. The Examiner went on to state that it would be obvious to quantify the mRNA levels since qRT-PCR is a known prior art technique. Finally, the Examiner stated that it is obvious that higher levels of a gene marker are correlated with a patient's prognosis.

The Examiner's reasoning is incorrect for several reasons. The Examiner apparently assigns a higher level of predictability to the field of the invention than is justified. Moreover, the Examiner's analysis does not accurately reflect the state of the art at the time the present patent application was filed. Particularly troublesome is the Examiner's belief that higher levels of gene markers are correlated with a patient's prognosis. The article *Quantitative real-time PCR: a powerful ally in cancer research* by S. Mocellin et al. (TRENDS in Molecular Medicine Vol.9 No.5 p. 189 - 195 May 2003) provides an assessment of the role of qRT-PCR in cancer research at the timeframe the present patent application was filed. (attached as Exhibit A). The Examiner's argument fails to appreciate the role that qRT-PCR played in cancer research prior to the filing of the present patent application. The Mocellin article explains that qRT-PCR was used for detection of minimal residual disease, tumor immunology, DNA copy-number measurement, and genomic mutation and polymorphism. In particular with respect to prognostic value, the Mocellin et al. article explains:

> Although the clinical utility of PCR-based MRD evaluation for hematological malignancies is well established, the experience with solid tumors is more limited. **Although some investigators have reported on the prognostic value of solid tumor [minimal residual disease] MRD detection at the molecular level [37], there is no general consensus on its clinical significance [38].** Unlike hematological malignancies, solid tumors are rarely

characterized by specific chromosomal translocations, and **tumor-specific markers are only expressed by some tumor types and in a relatively low percentage of cases**.

Mocellin et al., p. 192 (emphasis added)

The Mocellin et al. article clearly eviscerates the Examiner's contention that quantification of cancer related markers can be correlated with prognosis. This proposition is simply not supported by the prevailing knowledge in the state of the art at the time the present patent application was filed.

The deficiency of the prior art is further supported by the article *A Meta-analysis of Reverse Transcriptase–Polymerase Chain Reaction for Tyrosinase mRNA as a Marker for Circulating Tumor Cells in Cutaneous Melanoma* by H. Tsao et al. (ARCH DERMATOL/VOL 137, MAR 2001 p. 325-330) which describes an experiment relating to the prognostic value of the tyrosinase mRNA marker in melanoma patients. (Attached as Exhibit B). The Tsao article explains the lack of such utility in the following passages:

> Conclusions: The lack of data on the outcome of stage I, II, and III patients who were RT-PCR positive and **the low prevalence of RT-PCR positivity in patients with known stage IV disease limit the applicability of this test at this time**. Ongoing and future studies on a quantitative RT-PCR, amplification of multiple melanoma associated antigens, and use of the test as a prognostic indicator might improve the utility of this molecular serologic tool.
>
> Tsao et al. p. 325 (Emphasis added)
>
> Moreover, **the usefulness** and cost effectiveness of RT-PCR relative to other emerging serologic markers for melanoma, such as circulating S100 protein, 41-43 **remains to be established**.
>
> Tsao et al. p. 325 (Emphasis added)

The Tsao article undercuts two assumptions by the Examiner. The Examiner's assertion that more markers are better is undercut by the confused state of the art circa 2002. Applicants invite the Examiner to provide a publication or any evidence supporting this proposition. Moreover, the utility of qRT-PCR and quantification of mRNA in general was unproven at that time.

The article *Simultaneous Immunohistochemical Detection of Tumor Cells in Lymph Nodes and Bone Marrow Aspirates in Breast Cancer and Its Correlation With Other Prognostic Factors* by B. Gerber et al. (Journal of Clinical Oncology, Vol 19, No 4 (February 15), 2001: pp 960-971) also casts considerable doubt on the recognized utility of qRT-PCR in providing prognostic value circa 2001. (attached as Exhibit C). This article states with respect to breast cancer:

> Moreover, RT-PCR analysis is less expensive than currently available histopathologic examination techniques,[78] but it fails to distinguish benign from malignant epithelial cells.[67] Some cytokeratins (e.g., CK-19) **seem to have no diagnostic value as mRNA markers for micrometastases**; they are also expressed in blood and lymph nodes of healthy controls.
>
> Gerber et al., p. 968 (Emphasis added)

Many recent articles have shown qRT-PCR does not generally predict disease outcome in melanoma or other solid tumors. Moreover, prediction of disease outcome is useless unless a comparison to known prognostic factors for the tumor in question is performed in a multivariate statistical analysis as set forth in the present application. This proposition is supported in a recent article *Molecular Staging of Pathologically Negative Sentinel Lymph Nodes from Melanoma Patients Using Multimarker, Quantitative Real-time rt-PCR.* by J.M. Hilari et al. (Ann Surg Oncol. 2009 Jan;16(1):177-85), which concludes that "multimarker qRT-PCR analysis of SLNs did not correlate with disease recurrence. Our data support specific PAX3 splice variants but not GalNAc-T, PLAB or L1CAM as possible markers for melanoma

metastasis to SLNs." (Attached as Exhibit D). Another recent article *Detection of Tyrosinase mRNA in the Sentinel Lymph Nodes of Melanoma Patients is Not a Predictor of Short-term Disease Recurrence* by C. Tatlidil et al. demonstrates that tyrosinase markers are of no prognostic value - "detection of tyrosinase mRNA by RT–PCR alone does not appear to increase the likelihood of short-term disease recurrence." (Attached as Exhibit E). Another article demonstrating that RT-PCR of a single marker is of minimal prognostic value is *Sentinel Lymph Node Detection of Micrometastases of Melanoma in a Molecular Study* by VC Denninghoff et al. which observes that "[a]fter long follow-up period, molecular upstaging by tyrosinase RT-PCR failed to detect a subgroup of patients with an increased probability of recurrence." (Abstract attached as Exhibit F). Clearly, mRNA by RT-PCR alone does not appear to increase the likelihood of short-term disease recurrence. (See also *Prognostic Significance of Molecular Staging Study of Sentinel Lymph Nodes by Reverse Transcriptase-polymerase Chain Reaction for Tyrosinase in Melanoma Patients* by C. Mangas et al. (Annals of Surgical Oncology 13(7): 910-918). (Attached as Exhibit G).

At the very least, the Mocellin, Tsao, Gerber, and more recent articles indicate that the relevancy of gene markers in cancer diagnosis was uncertain at the time the present patent application was filed. The references demonstrate that not just any combination of cancer related gene markers would be useful for determining a melanoma patient's prognosis. The Federal Circuit recently explained:

> To differentiate between proper and improper applications of "obvious to try," this court outlined two classes of situations where "obvious to try" is erroneously equated with obviousness under § 103. In the first class of cases,
>> what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no

> direction as to which of many possible choices is
> likely to be successful.
> Id.
> In such circumstances, where a defendant merely throws
> metaphorical darts at a board filled with combinatorial prior art
> possibilities, courts should not succumb to hindsight claims of
> obviousness.

In re Kubin, (Fed. Cir. 2009)

The prior art in the context of the present invention exemplifies a situation in which there are a number of possible choices without an indication of which parameters are critical. In such a multifaceted disease such as cancer, blanket conclusions regarding the types of cancers, cancer gene markers, the expression levels of such gene markers, and the correlation of prognosis therewith are not obvious. This uncertainty is clearly realized by the Mocellin article's observation that "[a]lthough the clinical utility of PCR-based MRD evaluation for hematological malignancies is well established, the experience with solid tumors is more limited." Clearly, conclusions regarding hematological cancers cannot be imputed to solid tumors. The present invention provides a novel predicative method in which the levels of a plurality of mRNA transcripts have been found useful for evaluating the prognosis of a cancer patient – a melanoma patient.

Accordingly, for at least these reasons, claims 1-3, 5-7, 10 and 34-40 are allowable under U.S.C. § 103(a) over the analysis provided in the Interview.

Rejection of Claims 1-3, 5-7, 10 and 34
Under 35 U.S.C. § 102(b) Over Hoon

Claims 1-3, 5-7, 10 and 34 have been rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 6,057,105 issued to Hoon et al. ("Hoon"). Applicants respectfully traverse this rejection.

The present invention provides a method for predicting metastatic melanoma recurrence, metastatic melanoma-free survival, overall survival, or combinations thereof by quantifying the levels of the mRNA transcripts encoded by a panel of marker genes that include GalNAcT and PAX3:

> The present invention unexpectedly demonstrates that GalNAcT and PAX3 are new promising molecular marker for detecting occult melanoma cells. By using a large well-defined patient population with a significant clinical follow up, the present invention unexpectedly demonstrated that the quantitative detection of these mRNA markers in SLNs in the patients with early-stage melanoma has clinicopathological and prognostic utilities.
>
> Specification, p. 10, lines 20-25.

Independent claims 1 and 34 have been amended to recite "amplifying mRNA transcripts encoded by GalNAcT and PAX3 marker genes" and "detecting the levels of the mRNA transcripts encoded by the GalNAcT and PAX3 marker genes." These claims further recite "comparing levels of the mRNA transcripts encoded by the GalNAcT and PAX3 marker genes . . . from a second melanoma patient to levels of mRNA transcripts encoded by the GalNAcT and PAX3 marker genes . . . from the first melanoma patient." Therefore, the requisite quantification involves a step in which the RNA transcripts of the GalNAcT and PAX3 markers are amplified with their corresponding levels detected. Claims 1 and 34 now further recite a step in which levels of the mRNA transcripts between patients are compared for the purpose of making a prediction regarding "metastatic melanoma recurrence, metastatic melanoma-free survival, overall survival, or combinations thereof."

Hoon does not utilize techniques for quantifying the levels of RNA in the samples being analyzed. Instead, the predictions made by the methods of Hoon rely only on detection (and not quantification) to make certain predictions. This is evident from the fact that

quantitative reverse transcriptase PCR (qPCR) is not utilized in Hoon. Moreover, each method claim of Hoon includes a step of ""detecting the presence or absence of the nucleic acid targets." It should be appreciated that in claims 1 and 34, the phrase "detecting the levels of the mRNA transcripts" is a manifestation of the quantitative nature of the present invention. The assays used in the present invention are quantitative with significance being based on a cutoff above normal and other controls. Moreover, the quantitative nature of each assay point is based on a standard curve of known copy numbers of the same gene cDNA. This feature further differentiates the methodology of the present invention from other published techniques.

Hoon also fails to provide the step of "comparing levels of the mRNA transcripts . . . to predict predicting metastatic melanoma recurrence, metastatic melanoma-free survival, overall survival, or a combination thereof" as required by independent claims 1 and 34. The present invention demonstrates this by copy number through standard curve values and by comparison to normal specimen and reagent controls. The results are reported in binary form for statistical comparison (i.e., not as a continuous variable).

Moreover, Hoon fails to disclose utilization of the PAX3 marker gene as required by independent claims 1 and 34. Indeed, Hoon does not disclose the use of the PAX3 marker gene in evaluating metastatic cancer.

Accordingly, for at least these reasons, claims 1 and 34, as well as claims 2-3, 5-7, and 10 depending directly or indirectly from claim 1, are patentable under 35 U.S.C. 102(b) over Hoon, and reconsideration and withdrawal of this rejection are respectfully requested.

Rejection of Claims 1-7, 10, and 34
Under 35 U.S.C. § 103(a) Over Hoon and Johansson

Claims 1-7, 10, and 34 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Hoon in view of Johansson et al. (2000, Clinical Chemistry, 46(7): 921-927) ("Johansson"). Applicants respectfully traverse this rejection.

The deficiencies of Hoon with respect to the steps of "detecting the levels of the mRNA transcripts encoded by the GalNAcT and PAX3 marker genes" and "comparing levels of the mRNA transcripts encoded by the GalNAcT and PAX3 marker genes" to predict "metastatic melanoma recurrence, metastatic melanoma-free survival, overall survival, or combinations thereof" are set forth above. Johansson does not cure these defects of Hoon. Therefore, claims 1-7, 10, and 34 are also allowable over the present rejection.

The Examiner relies on Johansson to teach "a reproducible method comprising performing qRT-PCR to quantitatively detect mRNA markers of melanoma in biological samples" (Office Action, p. 6). The Examiner states the motivation for combining the teachings of Hoon and Johansson as being that "Johansson et al demonstrates that qRT *quantitatively* detects specific numbers of transcripts which are mRNA markers of melanoma in biological samples" (Office Action, p. 6). However, the combination of these references fails to appreciate the significance in quantifying and comparing the levels of the GalNAcT and PAX3 markers in predicting "metastatic melanoma recurrence, metastatic melanoma-free survival, overall survival, or combinations thereof" as required by independent claims 1 and 34.

Accordingly, for at least these reasons, claims 1 and 34, along with dependent claims 2-7 and 10, are patentable over the combination of Hoon and Johansson, and Applicant respectfully requests reconsideration and withdrawal of this rejection under 35 U.S.C. 103(a).

Rejection of Claim 35
Under 35 U.S.C. § 103(a) Over Hoon and Hatta

    Claim 35 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Hoon in view of Hatta et al. (Melanoma Research, August 1999, 9(4): 401-406) ("Hatta"). Applicants respectfully traverse this rejection.

    Claim 35 has been amended as set forth above to recite "isolating nucleic acid from a tumor-draining lymph node (TDLN) sample," support for which can be found throughout the application, for example, at p. 5, lines 30-32 of the specification. As Hatta is applied for its teaching of detecting circulating melanoma cells in non-sentinel lymph nodes (Office Action, p. 8), and claim 35 has been amended to be directed to TDLN samples, this rejection is now moot. For at least the reasons explained above in reference to independent claims 1 and 34, claim 35 is patentable over Hoon, either alone or in combination with Hatta. Therefore, Applicant also respectfully requests reconsideration and withdrawal of this rejection.

Rejection of Claim 35 Under 35 U.S.C. § 112

    Claim 35 has been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement for reciting isolating nucleic acid from a non-sentinel lymph node (NSLN) sample. As claim 35 has been amended to recite "isolating nucleic acid from a tumor-draining lymph node (TDLN) sample" as described above, this rejection is now moot.

New Claims

    New claims 36-40 have been added which include the steps of amplifying, detecting, and comparing mRNA transcripts encoded by a GalNAcT, PAX3, MAGE-A3 and MART-1 marker gene markers. For at least the reasons stated above, claims 36-40 are also patentable over the cited art.

Conclusion

In summary, Applicant believes that the claims meet all formal and substantive requirements and that the case is in appropriate condition for allowance. Accordingly, such action is respectfully requested. If a telephone conference would expedite allowance of the case or resolve any further questions, such a call is invited at the Examiner's convenience.

The Petition fee of $555.00 is being charged to Deposit Account No. 02-3978 via electronic authorization submitted concurrently herewith. The Commissioner is hereby authorized to charge any additional fees or credit any overpayments as a result of the filing of this paper to Deposit Account No. 02-3978.

Respectfully submitted,

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